The Role of Blood Transfusion in HCV Infection: A Study Testing Ab: RNA & Genotype

Prof. Dr. Waqar A. Al-Kubaisy* & Prof. Dr. Amjad D. Niazi

Faculty of Medicine, Universiti Teknologi Mara, Shah Alam, Selangor

**For reprint and all correspondence: Prof. Dr. Waqar A. Al-Kubaisy, Faculty of Medicine, Universiti Teknologi Mara, Shah Alam, Selangor*
Email: Waqar_abd@yahoo.co.uk

ABSTRACT

Introduction	Hepatitis C Virus (HCV) recently was identified as a major cause of post transfusion hepatitis world wide. To evaluate the role of blood transfusion on the prevalence of HCV infection, by testing antibody and RNA as well as the genotypes of HCV .Also to detect if Blood transfusion acts as unconfounding risk factor for HCV infection.
Methods	Sera from 3491 pregnant women were investigated for the presence of HCV antibodies (anti-HCV) by using third generation enzyme immunoassay (EIA-3) as screening test, followed by immunoblot assay (Lia Tek-III). In addition 94 sera of studied women were subjected to molecular analysis (at laboratories of Sorin BioMedica – Italy) for the detection of viral RNA and genotypes of HCV. Using RT-PCR & DNA Enzyme immunoassay (DEIA) method.
Results	Our study revealed, that seroprevalence rate of HCV specific Ab & RNA were significantly higher (16.32 %, 80% respectively) among women with a history of blood transfusion, compared to those (2.53%, 56.5%) with no such history P=0.0001, P=0.01. And there is a significant direct linear correlation between number of blood transfused and the seropositive rate of anti-HCV (r=0.7, p=0.046). Based on multivariate analysis, interestingly, this study confirmed that, blood transfusion significantly acting as unconfounding risk factor for acquiring HCV infection (Adjusted OR=1.938,95% C.I=1.646-2.28). And the risk of exposure is increases with increased number of blood transfused. Although, we found no significant association between, HCV genotypic distribution and history of blood transfusion. However, high proportion of women with a history of blood transfusion were harboring HCV genotype –4 or 1b, 50%,40%, resepctively.
Conclusions	Our study shows, evidence that, blood transfusion acts as unconfounding risk factor for acquiring and in a mode of transmission of HCV infection. Therefore strict screening of blood donor for HCV-Abs and / or RNA is highly recommended.
Keywords	HCV - mod of transmission - blood transfusion

INTRODUCTION

Hepatitis C (HCV) is an infectious disease affecting the liver caused by the hepatitis C virus (HCV) [1]. It is a systemic disease and patients may experience a wide spectrum of clinical manifestations ranging from an absence of symptoms to a more symptomatic illness prior to the development of advanced liver disease[2]. (HCV) is recognized as an important cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma[3, 4, 5, 6]. Interestingly, an estimated 270-300 million people worldwide are infected with hepatitis[7].

The hepatitis C virus is transmitted by blood-to-blood contact [8] according to Centers for Disease Control; hepatitis C virus is spread by exposure to large quantities of blood, either through the skin or by injection [9]. Therefore, blood transfusion, blood products, or organ transplantation prior to implementation of HCV screening is all risk factors for hepatitis C[10]. A cDNA clone from the hepatitis C virus genome was first isolated in 1989[10] and reliable tests to screen for the virus were not available until 1992. Therefore, those who received blood or blood products prior to the implementation of screening the blood supply for HCV may have been exposed to the virus[11]. Therefore, in the 1970s and 1980s, posttransfusional non-A, non-B hepatitis was the most frequent infection transmitted by blood and blood products, representing 80 to 90% of all cases of posttransfusional hepatitis)[12] and with the introduction of tests to detect these antibodies in the screening of blood units, there was a sharp drop in posttransfusional hepatitis[13, 14, 15, 16] . So there has not been a documented transfusion-related case of hepatitis C in the United States for over a decade, as the blood supply is vigorously screened with both EIA and PCR technologies[11, 17].

Anti-HCV antibodies indicate exposure to the virus, but cannot determine if ongoing infection is present. All persons with positive anti-HCV antibody tests must undergo additional testing for the presence of the hepatitis C virus itself to determine whether current infection is present. The presence of the virus is tested for using molecular nucleic acid testing methods, such as polymerase chain reaction (PCR) [18].

In Iraq, the prevalence of post transfusion hepatitis C is not known. Therefore we conducted our study among pregnant women to determine the prevalence of PTHCV using HCV anti bodies and HCV-RNA.

OBJECTIVES

The aims of this study are to assess the prevalence of post transfusion hepatitis C virus, to detect if blood transfusion acts as unconfounding risk factor for HCV infection, to identify the predominant HCV genotype(s) in post-transfusional hepatitis C virus.

METHODS

A sample of 3491 apparently healthy pregnant women, during third trimester was chosen randomly from 19 health care units located all over Baghdad. All pregnant women were interviewed by the same researcher, in order to avoid misclassification bias, using a brief specific questionnaire that focused on history of blood transfusion, date and number of blood unit transfused.

From each participant serum sample was obtained, and dispensed into two screw capped frozen tubes, stored at –20^{o}C and -70^{o}C for the antibody testing and molecular analysis respectively. Initial screening of HCV antibody was carried out, using third generation enzyme immunoassay (EIA-3). Then after, the positive results were confirmed further by the third generation immunoblot assay Lia-Tek III. Only reactive Lia-Tek III were considerable as positive serum samples. Furthermore, 94 serum samples (stored at –70^{o}C) were transferred by researcher to laboratories of Sorin Diagnostica (Sallugia, Italy) to be subjected to molecular analysis using the most recently advanced method RT-PCR and DNA enzyme immunoassay (DEIA) method .In which each sample was subjected to extraction of RNA followed by synthesis of complementary DNA (cDNA). After amplification of newly synthesized cDNA was carried out, finally detection of RNA and genotypes of HCV using DEIA method. This method is based on hybridization of the complementary (cDNA) with a single standard DNA probe coated on the wall of the micro titer plate wells with streptavidin-biotin band. Detection of hybridization is achieved by the use of anti-double stranded DNA monoclonal antibody. Finally, the result was detected by spectrophotometer. Depending on Simmond's Nomenclature for HCV genotypic classification that was proposed by international HCV collaboration group 1994, our classification was carried-on.

STATISTICAL ANALYSIS

Descriptive information is summarized as the total number of subject with and without history of blood transfusion along with number and percentage of subject with hepatitis C. for univariate analysis, the odds ratios (ORs) and 95% CIs were calculated from contingency tables. The OR represents the odds of HCV for subject with the risk factor (history of blood transfusion) relative to the odds of HCV for subject without risk factor. Multivariate analysis is summarized as an adjusted OR and 95% CI for dependent risk factor of blood transfusion identified by the multiple logistic regression models. All statistical tests were performed using $p < 0.05$.

RESULTS

A total of 387 units of whole blood were previously transfused to 151 mothers with a mean and range of 0.09 ±0.46, 1-8 units per woman. *HCV antibody test (Anti-HCV)* Lia Tek-III positivity was confirmed in 112 mother's sera. Therefore the overall anti-HCV prevalence was 3.21%. History of previous blood transfusion, was accompanied, significantly, by higher prevalence of anti-HCV 16.4% than their counter groups 2.5% (x^2=95.96 p=0.00001) (Table 1).

Table 1 Anti-HCV seropositivety (Lia-Tek III) and history of blood transfusion among 3491 pregnant women in Iraq

	History of Blood transifusion		
Anti-HCV Status	**Present**	**Absent**	**Total**
positive	28 (16.4)	84 (2.5)	112
Negative	143	3236	3379
Total	171	3320	3491

Interestingly, we found that with increased numbers of previous blood units transfused, was accompanied by significant increases of positive anti-HCV sero-prevalence, in a range of (7.55-58.33) (x^2=255.11, p=0.00001) (Table 2). Moreover, significant direct positive correlation was detected between increased HCV sero-prevalence and multiplicity of blood transfusion (r=0.74, p=0.046).

The association between potential risk factors of blood transfusion and HCV seropositivity was examined to develop a hypothesis on the modes of transmission of HCV. Our study revealed, that blood transfusion was strongly associated with HCV infection (OR=7.547, 95% C.I. 4.64-12.21). Calculating ORs separately for each unit of blood transfused verses non transfusion. The ORs showed steadily increasing values, with increasing amount of blood transfused. Therefore our study give evidence that strength of this association was significantly increases with increased amount of blood units transfused ranging OR=3.145-7.54 for 1-≥6 units of blood, with direct significant positive correlation r=0.98 p=0.0005.

Interestingly, using multivariate analysis, we found that blood transfusion was significantly acts as uncounfounding risk factor acting independently for acquisition of HCV infection adjusted OR=1.938 95%C.1 1.646-2.281 (Table 3)

Table 2 The relation between anti-HCV seropositive rate and number of blood unit transfused

	Blood units Transfused							
Anti HCV serostatus	0	I	II	III	IV	V	VI +	Total
Positive	84(2.53)	8 (7.55)	3(10)	7(58.33)	3 (50)	4(44.44)	3(37.50)	112
Negative	3236	98	27	5	3	5	5	3379
Total	3320(95.1)	106(61.98)	30(17.5)	12(7.02)	6(3.5)	9(5.26)	8(4.67)	3491

x^2=255.11, p=0.00001 r=0.74, S.E=16.68, p=0.046

Table 3 Crudr odds ratio (OR) of HCV infection correlated to number of blood unit transfused previously

Anti-HCV Positive	OR	95% CI	Significance
Transfusion Yes Vs No	7.543	4.640-12.21	S
Transfusion one unit Vs Nil	3.145	1.369-6.942	S
Transfusion two units Vs Nil	3.39	1.665-6.741	S
Transfusion three units Vs Nil	5.334	2.998-9.393	S
Transfusion four units Vs Nil	6.083	3.538-10.378	S
Transfusion five units Vs Nil	6.970	4.204-11.524	S
Transfusion six + units Vs Nil	7.543	4.640-12.21	S

r=0.98 S.E= 0.4 p=0.0005.

Regarding RT-PCR and DEIA method, for the detection of HCV-RNA and its genotyping, and to investigate the association between potential risk of blood transfusion and presence of HCV-RNA. Our study detected significantly higher HCV-RNA prevalence (80%) among 25 mothers with history of previous blood transfusion compared to (56.5%) those 69 with no such a history x^2=4.04 p=0.04

with marginal significance of OR =2.95 95% CI 0.97-10.39 (Table 4)

Table 4 HCV-RNA prevalence among 94 pregnant women in relation to history of blood transfusion

	History of Blood transifusion		
RT-PCR, HCV-RNA	**Present**	**Absent**	**Total**
Positive	20 (80)	39 (56.5)	59
Negative	5	30	35
Total	25	69	94

OR =2.95 95% CI 0.97-10.39 x^2=4.04 p=0.04 .

Genotyping of HCV

At least five HCV genotype/subtypes (1 1a 1b 3a or 4) were circulating among Iraqi population. Either in a single (1 1a 1b or 4) or mixed (1+4, 1b+4; 3a+4) pattern of infection. The predominate HCV genotype among women with history of blood transfusion were HCV-4 followed by HCV-1b. Interestingly, HCV-3a was absent in sera of women with history of blood transfusion. However, significant evidence of association between HCV genotypes and potential risk factors of previous blood transfusion was detected from our study.

Table 5 HCV genotypic distribution according to history of blood transfusion

	HCV Genotype &Subtypes							
Blood tranfusion	1	1a	1b	4	1 & 4	1b & 4	3a & 4	Total
Present	3	3	4	5	1	4	-	20
Absent	2	10	6	8	2	6	3	37
Total	5	13	10	13	3	10	3	57

x^2=2.32 , p=0.8

DISCUSSION

Post transfusion hepatitis is a major public health problem world wide. HCV was characterized as parentally transmitted included blood and blood product[15, 19, 20, 21, 22] and HCV was identified as a major cause of post transfusion hospital (PTH) [22, 23]. Therefore the parental spread of HCV, documented as unquestionable[24]. This issue was confirmed by our study in which an evidence was presented that blood transfusion is a major and unconfounding risk factor for acquiring HCV infection among Iraqi pregnant women based on univariate, multivariate and logistic regression correlation (OR=1.936 95% C.I 1.64-2.28) adjusted OR= 7.54, 95% C.I.=4.6-12.7, r=0.98, p=0.00056 respectively. PTHC virus antibody was found (16.37%) 6 times significantly greater than the counter group, and this values is less than (22%) that of French pregnant women[25], but higher than (13%, 3.7%) of American and Italian women respectively[26, 27]. The most important finding of our investigation is the unconfounding risk factor of blood transfusion which was consistent with several studies among pregnant women in Mexico and Spain[21,28] and population other than pregnant women[24, 29, 30, 31] were OR, (95% C.I.) as 9.6 (4.4-20.7), 3.2 (1.4-7.3), 2.4, 4.07 (2.9-5.6), 2.13 (1.32-2.2) respectively. This variation may be due to the different assay system, HCV genotypes, stage of disease or strict screening strategy in blood donors selection as that in Swedish population (anti-HCV0.026) [31]. Although anti-HCV prevalence among Iraqi blood donors was about 0.5%[32], unfortunately, blood screening for HCV was established since late 1995. This may partly explain the high prevalence of PTHC or it may be that blood units was collected during the window period of HCV seroconversion[33]. High parity[34] and age of mother, however on the contrary, failed to give any evidence for the significance of blood transfusion as a risk for transmission of HCV, neither by multivariate, nor by univariate[35, 36, 37]. Moreover our significant positive linear correlation between positive HCV and increased number of blood units transfused (r=0.7 P=0.046) was in contrast to other result[38].

The astounding finding, that we confirmed the direct significant dose response correlation between number of blood unites transfused and increased risk of exposure to HCV. Interestingly, it was started from the first blood unit transfused (OR=3.14 95% CI 1.3-6.94) onward disagreement to other[31]. To our knowledge this is the first attempt world wide to clarify without any doubt, the impact of number of blood units transfused on the acquisition of HCV among pregnant women. However, several authors reported such finding among population other than pregnant women[30, 39, 40]. Moreover, with history of blood transfusion our pregnant women showed significantly, higher rate

of HCV–RNA which was compatible with other finding[22, 39, 41].This mean that such women were more likely to be viremic than their counter group. Regarding to genotypes and its relation to PTH, in consistent to study done[42], we failed to detected such relation which was in contradict other studies, they found that the most predominant PTHC infection genotype was HCV-1b[43,44,45,46]. However we detected that genotype 4 or 1b (in a mixed or single pattern of infection) were the predominant circulating among PT HC women. A striking observation, we found that with increased number of blood units transfused ($\geq$ 3) the possibility of HCV genotypes co-infection increases (data not shown). This confirmed other finding[47, 48].

CONCLUSION

Post transfusion hepatitis is a major public health problem in Iraq is the blood transfusion acts as unconfounding risk factor for aquasition of HCV We gave evidence of direct significant dose response correlation between number of blood unites transfused and increased risk of exposure to HCV.which was started from the first blood unit transfused onward . The predominant HCV genotype were HCV-4 and 1b (in a mixed or single pattern of infection) among PT HC in Iraq Therefore strict screening of blood donor for HCV-Abs and / or RNA is highly recommend.

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